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• 英文關鍵字	ketamine；GHB；blood-brain barrier；cerebrovascular endothelial cells；；；；		
• 中文摘要	研究目的：Ketamine 和 GHB 是常被濫用的成癮藥物之一，然而其對腦血管血腦障壁(blood-brain barrier)的作用並不清楚，所以本研究旨在探討 ketamine 和 GHB 對血腦障壁以及其主要組成腦內皮細胞(cerebrovascular endothelial cells)的影響，以及其可能的作用機轉。 研究方法：腦內皮細胞製備自小鼠大腦微血管，並以免疫細胞染色法加以鑑定。細胞毒性以細胞型態(morphologies)、細胞膜完整性(membrane integrity)和細胞存活率(cell viability)做為評估方法。流式細胞儀分析法(flow cytometry)用於評估細胞是否發生凋亡(apoptosis)作用。腦內皮細胞形成 tight junction 結構的通透性(permeability)，以 dextran-FITC 螢光分析法加以測定。小鼠(mice)以腹腔注射方式打入 ketamine 或 GHB 之後，血腦障壁的完整性分別以 Evan's blue、免疫細胞染色法和共軛焦顯微鏡分析法予以量測。 主要發現：本計劃已成功自小鼠大腦微血管分離出腦內皮細胞，而且其純度大於 90%。當以 ketamine 和 GHB 處理腦內皮細胞之後，此兩種濫用藥物皆會減少細胞數目、干擾細胞膜完整性、並會降低細胞存活率。Ketamine 和 GHB 亦會導致細胞發生凋亡現象。腦內皮細胞形成 tight junction 的結構，會因 ketamine 和 GHB 的處理而增加其通透性。當小鼠以 ketamine 處理之後，其腦部微血管血腦障壁的完整性會被破壞，但是 GHB 則沒有此種作用。Factor VIII 和 occludin 的免疫細胞染色分析發現，ketamine 會降低腦組織的腦內皮細胞數目，並會干擾 occludin 形成 tight junction 聚合結構的能力。然而，GHB 卻不會有此一作用。 結論及建議事項：此一研究顯示，在 in vitro 的研究模式中，ketamine 和 GHB 在較高濃度下，皆會造成腦內皮細胞的死亡，可能部份是透過細胞凋亡的機轉。ketamine 和 GHB 都會破壞腦內皮細胞形成的 tight junction 結構，然而只有 ketamine 在 in vivo 的動物實驗中被證實，其會經由干擾 occludin 形成 tight junction，進而破壞血腦障壁的完整性。所以，若長期濫用此兩種藥物，可能會增加腦血管血腦障壁損傷的風險度，並進而造成其後發生的腦神經組織的		

傷害。

Objectives: Ketamine and GHB are commonly used as drugs of abuse. Meanwhile, it was still unknown if these two drugs can damage cerebrovascular functions, especially the blood-brain barrier. Thus, this study was aimed to evaluate the effects of ketamine and GHB on the blood-brain barrier and cerebrovascular endothelial cells, the major components of the blood-brain barrier. Methods: Cerebrovascular endothelial cells were prepared from mouse brain tissues, and were identified using immunocytochemistry. The toxicities of ketamine and GHB to cerebrovascular endothelial cells were determined by analyses of cell morphologies, membrane integrity, and cell viability. Apoptotic cells were quantified using flow cytometry. Permeability of cerebrovascular endothelial cell-forming tight junction was analyzed using dextran-FITC as the probe. Mice were intraperitoneally injected with ketamine and GHB. The integrity of the blood-brain barrier was assayed using Evan's blue as the dye. Confocal microscopy was carried out to analyze the occluding-form tight junction. Results: We have successfully isolated cerebrovascular endothelial cells from mouse brain tissues, which were identified by immunocytochemical analyses of vimentin and Factor VIII. Exposure of cerebrovascular endothelial cells to ketamine and GHB decreased cell numbers, interrupted the membrane integrities, and induced cells death. Both ketamine and GHB could induce cerebrovascular endothelial cells to undergo apoptosis. Treatment of cerebrovascular endothelial cells with ketamine and GHB increased the permeability of the cell-forming tight junction. After administering ketamine into mice, the blood-brain barrier was interrupted. Results by confocal microscopy revealed that ketamine decreased the number of cerebrovascular endothelial cells and broke-down the occludin-constructing tight junction. Meanwhile, GHB had not such effects. Conclusion and suggestion: This study shows that ketamine and GHB can lead to death of cerebrovascular endothelial cells via an apoptotic mechanism. Both ketamine and GHB can interrupt the tight junction, which is constructed by cerebrovascular endothelial cells. However, only ketamine has its toxicity to the blood-brain barrier in mouse brain through suppression of occludin polymerization. This study suggests that when ketamine and GHB are abused for long periods, they may induce dysfunction of the blood-brain barrier, then leading to brain injuries.

• 英文摘要